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QUANTITATIVE MICROBIOLOGY OF THE SCALP IN NON-DANDRUFF, DANDRUFF, AND SEBORRHEIC DERMATITIS

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The composition of the scalp microflora was assessed quantitatively in normal individuals and in patients with dandruff and seborrheic dermatitis, disorders characterized by increasing scaling. Three organisms were constantly found: (1) Pityrosporum, (2) aerobic cocci, and (3) *Corynebacterium acnes*.

Pityrosporum (mainly *Pityrosporum ovale*) made up 46% of the total microflora in normals, 74% in dandruff, and 83% in seborrheic dermatitis. The geometric mean number of organisms per cm^2 in non-dandruff subjects was 5.04×10^5; 9.22×10^5 in dandruff subjects; and 6.45×10^5 in those with seborrheic dermatitis. The cocci were dominantly Baird-Parker type SII and no quantitative or qualitative change occurred in the scaling disorders. *C. acnes* comprised 26% of the flora on the normal scalp, 6% in dandruff, and only 1% in seborrheic dermatitis.

These results differ significantly from previous reports which describe a much more complex microflora and suggest an etiologic role for microorganisms in dandruff.

Although it is now 100 years since Malassez declared a yeast-like organism, Pityrosporum, to be the cause of dandruff, the matter is still undecided [1]. Over the years, there has been a fitful stream of nonquantitative studies aimed at examining the relationship between various scaling scalp disorders and microorganisms. Most of these are now mainly of historical interest, the methods for the most part having been inadequate to the task. What comes down to use is a large inventory of microorganisms that have been recovered from the scalp. The isolation of one or two colonies contributes to incidence figures, but may tell more about contaminants from the environment than about the organisms actually living and multiplying at this site.

The scalp is a lush garden for skin organisms. High densities of sweat glands keep the surface moist. Rapid epidermal turnover assures a steady supply of nutrients while numerous, large sebaceous glands furnish abundant lipids that can be used directly or indirectly for nourishment. Consequently, the population of microorganisms is considerably denser than on glabrous skin.

Our objectives have been to characterize the normal microflora qualitatively and quantitatively and to determine the changes that occur in two common scaling disorders: dandruff and seborrheic dermatitis.

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MATERIALS AND METHODS

Subjects

There were three sources of patients: (1) adult male prisoners (Philadelphia County Prison); (2) middle-aged volunteers from the Philadelphia Home for the Aged (Riverview Home); (3) out-patients from the Dermatology Clinic of the Hospital of the University of Philadelphia.

Three groups were recruited: 126 with dandruff, 63 with seborrheic dermatitis, and 112 normals. Normal subjects were selected on the basis of no evidence of history of scaling on the scalp and no evidence of any inflammatory process such as atopic dermatitis, seborrheic dermatitis, psoriasis, or ichthyosis elsewhere on the body. Although the mean age in each group was 46, the distributions were different. The proportion of elderly persons was greater in the seborrheic dermatitis group, reflecting an increased incidence of this disorder with age. The patients with seborrheic dermatitis were almost all white. The dandruff subjects were mainly black, and the out-patients were mainly white adults. In all, there were 188 black subjects and 118 whites. We have considered black and white together since we have found no important racial differences regarding the parameters measured. Since there were only 15 females, sex was not separately analyzed. It is apparent, however, that the groups were not evenly matched with regard to age and race. Informed consent was obtained from all subjects before they were sampled.

Criteria for Recruitment

We define dandruff as noninflammatory, increased scaling. Prospective subjects were graded 4 days after a bland shampoo using a 10-point scale in which Grade 5 signifies moderate dandruff. Normals had grades of 3 or less; only those with grades of 5 or more were considered to have dandruff [2]. Seborrheic dermatitis was not diagnosed unless the picture was classic inflammatory

scalp scaling accompanied by typical greasy lesions of the face and trunk.

Sampling Technique: The Detergent Scrub

We have utilized this procedure extensively to measure the quantity of horny cells produced per unit time (4 days post shampooing) and to quantify the resident microflora [3]. A glass cylinder with an area of 3.8 sq cm is held firmly to the skin and 1 ml of wash solution, 0.1% Triton-X-100 in 0.075 M phosphate buffer (pH 7.9), is pipetted into the cup. The surface is vigorously scrubbed with a sterile, blunt Teflon "policeman." The wash fluid is aspirated and a second 1 ml is pipetted into the cup and scrubbed for an additional 1 min. The 2 washes are then pooled. Further dilutions are made with half-strength wash solution to prevent reaggregation of particles.

Culture Methods

For counting, 10-fold dilutions of wash fluid to 10^{-4} were made and 0.025 ml dropped onto the following solid media with a 0.2-ml bacteriologic pipette: (1)Trypticase Soy Agar (TSA) (Baltimore Biological Labs) with 0.5% yeast extract added, a general medium for aerobic organisms; (2) TSA with lecithin and Tween-80 (Baltimore Biological Labs) to enhance the growth of lipophilic diphtheroids; (3) Littman-Oxgall agar (BBL) for the growth of yeasts; (4) CYLG, a modified Marshal and Kelsey agar for anaerobic growth of *Corynebacterium acnes*, which was also used for aerobes since it enhances pigment formation, facilitating identification [4].

Counts were made 3 days after aerobic incubation at 35°C. Bacterial groups were identified after a further 4 days at room temperature. Anaerobic plates were incubated at 35°C for 7 days in an atmosphere of 90% nitrogen and 10% CO_2. To remove traces of oxygen, steel wool moistened with saturated copper sulfate was placed in each jar.

Taxonomic Criteria

1. *C. acnes.* Anaerobic gram-positive rods. *C. acnes* was further divided into Groups I and II on the basis of colonial morphology and susceptibility to bacteriophage [4].
2. *Staphylococcus aureus.* Coagulase-positive, catalase-positive, gram-positive cocci.
3. Coagulase-negative cocci. Catalase-positive, gram-positive cocci subdivided according to the scheme of Baird-Parker as modified by us [5].
4. Lipophilic diphtheroids. Diphtheroids showing markedly enhanced growth in the presence of Tween-80.
5. Other aerobic diphtheroids.
6. Gram-negative rods; no further identification.
7. Yeasts, mainly Candida species; no further identification.
8. Aerobic spore formers.
9. Pityrosporum. Since Pityrosporum cannot be quantitatively recovered by culturing, direct cell counts were made. A volume of 0.2 ml of the scrub sample was pipetted onto a 9-mm circular coverslip on a cork support. After drying, triple-strength Giemsa stain (Fisher) was added for 1 hr, the coverslips rinsed in distilled water, then dried and mounted. Yeast cells were counted under oil immersion using an eyepiece grid. Oval-shaped cells were identified as *Pityrosporum ovale*, spherical ones as *P. orbiculare*. Hyphal outgrowths, usually short, were termed filamentous forms.

Counting and Cytology

After processing for microbiologic analysis, the remainder of the original scrub sample was diluted 5-fold, greater when scaling was heavy, and corneocyte counting was performed according to the method of McGinley et al [6]. Two drops of 2% crystal violet were added and the sample was mechanically shaken for 30 sec. The cells were then counted in a Fuchs-Rosenthal hemocytometer. Nucleated horny cells (nucleocytes) were separately appraised using the high dry objective.

Statistics

Because both corneocytes and microorganisms are log-normally distributed, the values were transformed into logarithms per sq cm. The data were subjected to the one-way analysis of variance and significance assessed according to the method of the "least significant difference" (LSD).

RESULTS

Corneocyte Counts

The geometric mean in non-dandruff was 487,000 cells per sq cm compared to 780,000 in dandruff and 706,000 in seborrheic dermatitis (Tab. I, Fig. 1). A statistically significant difference existed between non-dandruff and the two scaling disorders ($p < 0.01$ in both cases) but not between dandruff and seborrheic dermatitis ($p > 0.05$).

Nucleocyte Counts

The number of nucleated cells varied significantly in each group (Tab. I, Fig. 1). The arithmetic percentage range in non-dandruff was 0 to 6% of the total count with the geometric mean count being 3,744 cells per sq cm; in dandruff, the arithmetic percentage range was 1 to 18% with a geometric mean of 25,240; in seborrheic dermatitis, the range was 4 to 23% with a geometric mean count of 76,000 nucleated cells per sq cm.

Microbiology

Aerobes. There were no significant differences in total numbers, the means being 277,100 per sq cm for non-dandruff, 249,000 per sq cm for dandruff, and 139,100 for seborrheic dermatitis. Figure 2 and Table I show the organisms isolated and their proportions in each group. Coagulase-negative cocci were isolated from all subjects and constituted about 93% of the aerobic bacteria. *S. aureus* was uncommon in normals and in dandruff, making up a tiny fraction when present. By contrast, it was recovered in about one-fifth of seborrheic dermatitis cases, averaging about 32% of the total flora when present.

Baird-Parker typing was done on the cocci from 79 subjects (Tab. II). SII was by far the most frequent, being recovered from 95% of scalps. Micrococcus type III (M3) was present in about 50%, while SVI, MI, and M7 were present in 25% each. The distribution of the coccal types was the same in the three groups.

TABLE I. *Microbiology and cytology*

Means covered by bars are not significantly different from one another by analysis of variance.

		Non-dandruff	Dandruff	Seborrheic dermatitis
Subjects		112	126	63
Corneocytes	GM[a]	487 700	780 000	706 000
	SD[b]	0.22	0.21	0.20
Nucleocytes	GM	3 744	25 240	76 000
	SD	1.53	0.92	0.55
Pityrosporum	GM	503 900	922 100	664 500
	SD	0.49	0.56	0.59
Aerobes	GM	277 100	249 300	139 100
	SD	0.85	1.26	1.20
C. acnes	GM	296 300	75 500	7.551
	SD	1.35	1.58	1.83

[a] Geometric mean per cm^2

[b] Standard deviation (expressed logarithmically)

Lipophilic diphtheroids were the next most prevalent, being present in about 50% of scalps, but the numbers were small, contributing about 10% of the aerobic flora. Other aerobic diphtheroids, gram-negative rods, aerobic spore formers and yeasts other than Pityrosporum or *Candida albicans* were only occasionally isolated in small numbers.

C. acnes. The three groups differed markedly regarding the anaerobic population. The values were highest in non-dandruff, 296,300 per sq cm; next highest in dandruff, 75,500 per sq cm; and least, indeed very low, in seborrheic dermatitis, 7,551 per sq cm. Group I *C. acnes* was isolated in practically every case (98%). Group II was recovered in 4% of non-dandruff, 8% in dandruff, and 11% in seborrheic dermatitis (Tab. III).

Pityrosporum. These yeast-like fungi were very abundant. The values were least for non-dandruff, 503,900 per sq cm. While increased in seborrheic dermatitis, 644,500 per sq cm, statistical significance was not quite achieved. In dandruff, the yeasts were significantly higher than in non-dandruff, 922,100 per sq cm ($p < 0.05$). No significant difference was detected between dandruff and seborrheic dermatitis (Tab. I, Fig. 2).

P. ovale was present in virtually all cases. *P. orbiculare* was present in approximately 80% of subjects, the groups not differing significantly. In occasional subjects, *P. orbiculare* predominated. Filamentous forms occurred in 6% of non-dandruff, 23% of dandruff, and 27% of seborrheic dermatitis (Tab. III).

When the geometric mean counts of the three major components of the scalp microflora are combined, the yeast-like organisms comprise 46% of the total scalp flora in non-dandruff, 74% in dandruff, and 82% in seborrheic dermatitis.

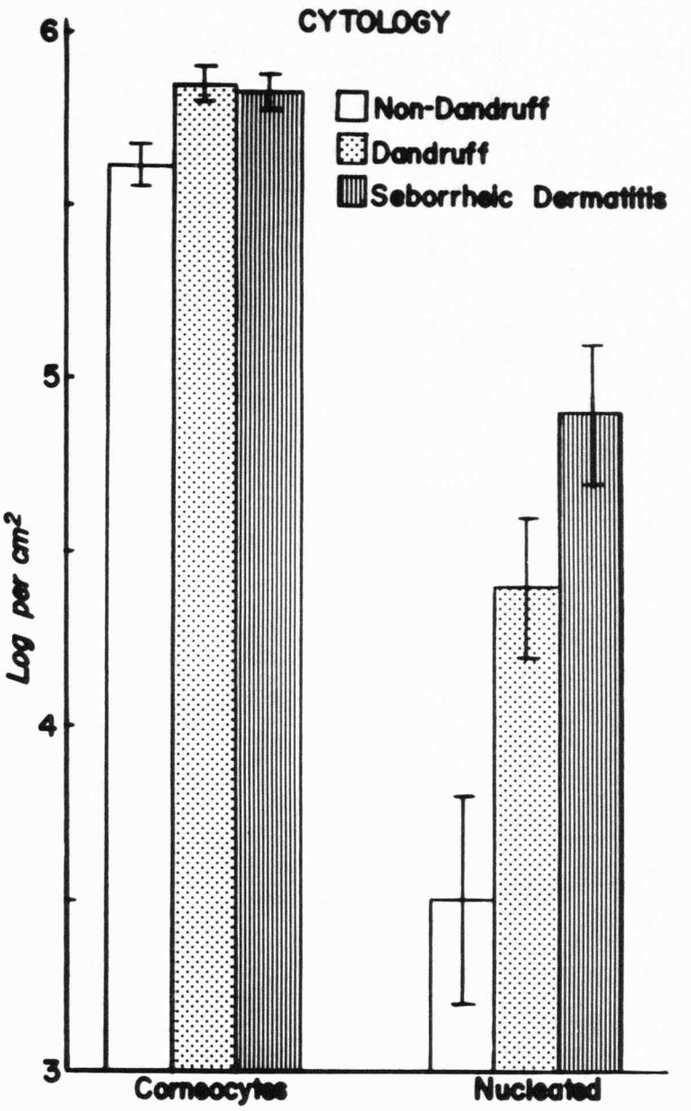

FIG. 1. Quantitative determination of the number of desquamating cells (corneocyte count) and the number of nucleated cells (nucleocyte count) in the three groups. Bars represent the 95% confidence limits of the standard error of the mean.

DISCUSSION

Our aim has been to characterize the microflora of the normal and diseased scalps. We thought that such knowledge might define what role microorganisms play in dandruff and in seborrheic dermatitis.

The microflora of the normal scalp is quite simple. Three groups of organisms are always found: (1) Pityrosporum, (2) *C. acnes*, and (3) aerobic cocci. These are residents in most body regions. The low incidence and numbers of aerobic diphtheroids make the scalp unusual. Another exceptional feature is the high incidence of Micrococcus type III in about 50% of scalps, though in small numbers.

The same triad of organisms occurs in dandruff and in seborrheic dermatitis, but the proportions are different. *C. acnes* was particularly interesting in this regard. It comprised about a third of the microbiotica of the normal scalp, about 6% in dandruff and only 1% in seborrheic dermatitis. We take these drastic decreases in numbers to reflect increased blood flow from inflammatory changes. *C. acnes* is a strict anaerobic and even a slight

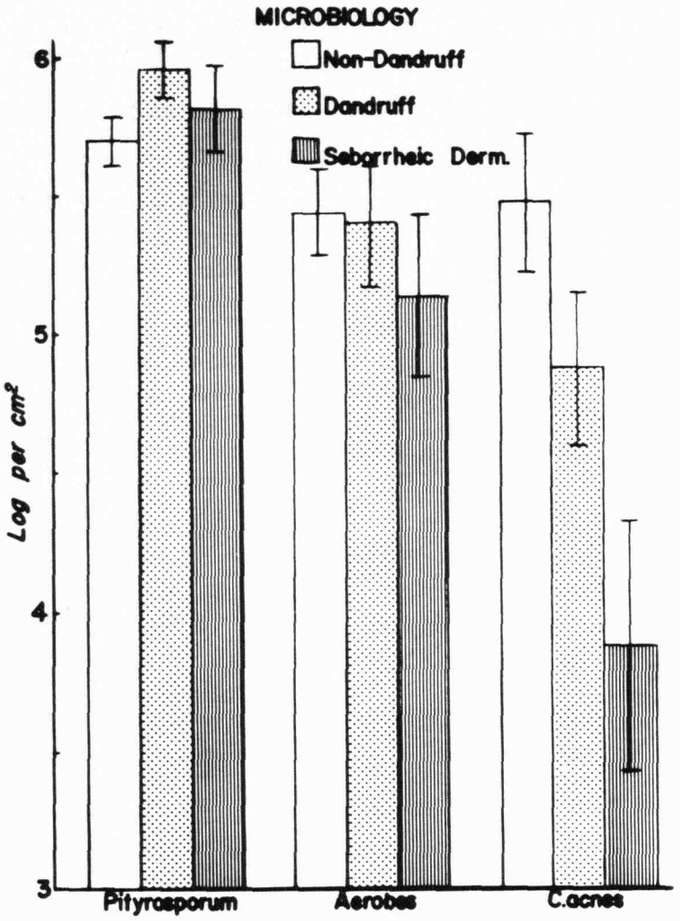

FIG. 2. Quantitative microbiology of the scalp in seborrheic dermatitis, dandruff, and non-dandruff. Bars represent the 95% confidence limits of the standard error of the mean.

TABLE II. *Baird-Parker typing of cocci: % prevalence*

	Non-dandruff	Dandruff	Seborrheic dermatitis	All groups
SI	4	3	21	9
SII	93	82	83	86
SIII	0	0	0	0
SIV	7	0	0	3
SV	11	12	6	10
SVI	29	9	33	22
M1	25	6	33	19
M2	7	9	17	10
M3	50	82	36	65
M4	0	0	0	0
M5	4	3	0	3
M6	7	12	22	13
M7	21	55	44	41
M8	7	3	0	4
Total subjects	28	33	18	79

increase in O_2 tension from dilated vessels would antagonize growth. The inflammatory aspects of seborrheic dermatitis are clinically evident as erythema and exudation. In dandruff, as we shall show elsewhere, there are numerous microfoci of inflammation in the papillary dermis, especially in papillae adjacent to follicles. Anaerobes congregate in the infundibulum, and would be especially vulnerable to a slight increase in oxygen supply. It would seem that the density of *C. acnes* varies inversely to the degree of inflammation.

The percentage of nucleocytes varied inversely with *C. acnes*. Nuclei are retained when epidermal turnover is accelerated, a response always associated with inflammatory change. The percentage of nucleocytes was very low in the normal scalp, with an average of 3%; this increased to 9% in dandruff and to 13% in seborrheic dermatitis. Nucleocytes and *C. acnes* could, in fact, serve quite well as diagnostic discriminants in scaling disorders. The same relationships hold for psoriasis of the scalp (unpublished observations).

Group I *C. acnes* greatly predominated over Group II in all cases. Group II could be isolated in only a minority of cases and its numbers were low.

There was an inverse relationship between *C. acnes* and Pityrosporum. As *C. acnes* declined, the relative proportion of Pityrosporum increased from about 43% in normals, to 75% in dandruff, and about 80% in seborrheic dermatitis.

It is somewhat surprising that the aerobes did not increase as scaling became more abundant; indeed, these tended to diminish. In atopic dermatitis and psoriasis of the glabrous skin, resident cocci do increase considerably [8,9]. Perhaps this is prevented on the scalp because Pityrosporum normally dominates the region and multiplies very rapidly when scaling increases. Pityrosporum makes up almost 50% of the resident microflora, increasing to 75% in dandruff and about 80% in seborrheic dermatitis. It is very abundant on the normal scalp, about half a million organisms per sq cm. *P. ovale* was the dominant form in the vast majority of subjects. We found rudimentary filamentous forms in 6% of normals, 23% of patients with dandruff, and 26% of patients with seborrheic dermatitis. Filamentous forms did not occur with enough frequency or in enough quantity to imply a pathogenic role.

Regarding organisms other than residents, only *S. aureus* deserves passing mention. Its incidence was 4% in normal scalps; 3% in dandruff. The numbers were low, comprising 3% of the aerobic flora in non-dandruff and 6% in dandruff. *S. aureus* was isolated from 21% of seborrheic dermatitis cases and comprised 32% of the flora when present, a significant increase. On the face of it, *S. aureus* is not a causative factor in this disease, although in neglected cases it may induce a secondary pyoderma. Its presence signifies inflammatory change. It occurs in high numbers in all cases of atopic dermatitis and in about 30% of psoriatic lesions [8,9].

The data presented here cannot be constructed to argue for or against an etiologic role for bacteria or yeast-like organisms in dandruff. Other kinds of experiments involving the selective eradication of species will speak to that question.

Our results must be compared to those of other workers. The most complete recent microbiologic study is that of Roia and Vanderwyk [10]. Their findings are almost completely antithetic to our own. They identified "225 resident isolates" in-

TABLE III. *Incidence and composition of microflora*

Microbial group	Non-dandruff		Dandruff		Seborrheic dermatitis	
	% Prevalence	% of Flora[a]	% Prevalence	% of Flora[a]	% Prevalence	% of Flora[a]
Aerobes						
S. aureus	4	3	3	6	21	32
Coagulase-negative cocci	100	95	100	97	100	86
Lipophilic diphtheroids	55	9.7	48	5	59	14.9
Other aerobic diphtheroids	1	1.1	5	0.8	24	7.6
Gram-negative rods	6	0.01	6	0.01	14	1.8
Yeast	0	0	2	0.003	2	0.6
Aerobic spore formers	1	0.1	1	0.001	3	0.99
Anaerobes						
C. acnes Group I	98	99.3	97	93.7	90	97.7
C. acnes Group II	4	17	8	23	11	19
Pityrosporum						
P. ovale	98	82	100	91	100	86
P. orbiculare	78	18.1	77	9.2	81	14.1
Filamentous forms	6	0.001	23	0.002	27	0.003
Total subjects	112		126		63	

[a] Percent of flora when present.

cluding 30 yeasts, 143 molds, 44 bacteria, and 8 actinomycetes in 28 dandruff patients and 24 normal subjects. Surely the term "resident" is unfortunate. So vast a flora must derive from the environment and represent transients which are not residing on the skin. Hair is an efficient trap for particles, including microorganisms. It is inevitable that a few cells of virtually all the organisms which share the environment with man would be recovered by systematically sampling scurf as these workers did. They found *P. ovale* in 96.4% of dandruff cases but in only 75.0% of normals. We found a 100% incidence in dandruff, while in normals Pityrosporum was present in 110 of 112 samples—a greater than 98% incidence. We believe the incidence of 100%, and that technical errors account for occasional cases in which Pityrosporum is not found. Roberts [11] found *P. ovale* in 97 of 100 cases, but like ourselves doubted that Pityrosporum was ever completely missing. Our findings of filamentous forms in about 6% of normal scalps is almost identical to Roberts's value of 3%. We do not exclude the possibility that the filaments might arise from *P. ovale* as well as *P. orbiculare*.

We are completely baffled by Roia and Vanderwyk's statement that "resident bacteria were found in 57.1% of dandruff subjects and in 25% of those without dandruff." The most prevalent species was *Bacillus subtilis* (15.4%). *S. epidermidis* was found in only 45%. We believe that the incidence of cocci in normal or diseased scalps is 100%; failure to find cocci requires an explanation. Finally, we emphatically disagree with Roia and Vanderwyk's conclusion that microorganisms are a factor in dandruff because "almost twice as many species of organisms were found in dandruff scalps as compared to normals." Their figures refer to prevalence rates and not to the quantity of organisms on the scalp. The only organism which is significantly increased in dandruff is the yeast, Pityrosporum.

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